Reprinted from the American Journal of Physiology.

VOL. XVII. — NOVEMBER 1, 1906. — NO. III.

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[FROM THE PHYSIOLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.]

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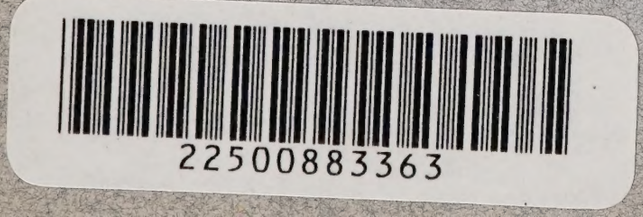

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NOTE UPON THE PRESENCE OF AMIDO ACIDS IN THE BLOOD AND LYMPH AS DETERMINED BY THE β NAPHTHALINSULPHOCHLORIDE REACTION.

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SINCE the introduction of the β naphthalinsulphochloride reaction by Fischer and Bergell,[1] it has been used by a number of observers to test for the presence of amido acids in the urine. The result of this work has been to show the probable normal occurrence in the urine of glycocoll at least, partly in a free form and partly combined.[2] Von Bergmann[3] has also demonstrated by the same reaction the probable existence of an amido body of unknown composition in the normal blood of dogs, and in human blood in a case of acute yellow atrophy. In the course of some work upon the composition of blood with special reference to the absorption of the products of proteid digestion, the present author has obtained the β naphthalinsulphochloride reaction from normal blood by a simple method. Instead of removing the proteid incompletely by heat coagulation according to the method used by von Bergmann, the amido bodies were separated by dialysis. For this purpose the convenient collodium membrane was used in place of parchment paper. This membrane, first employed, I believe, in pathological experiments at the Pasteur Institute, can be made very easily and be given any desired form. It dialyzes much more rapidly than parchment paper for all readily dialyzable substances, but is impermeable to the usual proteids of blood unless the dialysis is con-

[1] Fischer and Bergell: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 3779.

[2] Ignatowski: Zeitschrift für physiologische Chemie, 1904, xlii, p. 371; Embden and Reese: Hofmeister's Beiträge, 1905, vii, p. 411; Forssner: Zeitschrift für physiologische Chemie, 1906, xlvii, p. 15; Abderhalden and Schittenhelm: *Ibid.*, 1906, xlvii, p. 339, and Samnely: *Ibid.*, 1906, xlvii, p. 376.

[3] Von Bergmann: Hofmeister's Beiträge, 1904, vi, p. 40.

tinued for a long period. In the present experiments the membrane was constructed in the form of a flask with a capacity of about 250 c.c. For this purpose one takes a 5 to 6 per cent solution of gun cotton in a mixture of equal parts of absolute alcohol and ether. Some of the solution is poured into an Erlenmeyer flask of the desired capacity, and is then turned out while the flask is kept in slow rotation, so as to form an even layer of the adherent solution over the entire wall. The flask is drained carefully to remove the superfluous collodium, and is rotated slowly in the hands for several minutes until the solution adherent to the walls has hardened superficially. The flask is then filled with water; the membrane of collodium is separated at the mouth of the flask by means of a knife, and is gently detached from the walls of the flask for a distance by means of a blunt glass rod or spatula. After emptying the flask the entire membrane may then be removed conveniently by allowing a stream of water to flow in between the membrane and the glass wall. The membrane thus loosened may be pulled out without danger of rupturing. It presents the form of the Erlenmeyer flask, and may be kept in distilled water until used. In using these flasks to dialyze blood a glass tube 30 to 40 cms. in length was cemented into the mouth with collodium solution and firmly tied. By this means one prevents the overflow of the flask which would otherwise occur in consequence of the permanent osmotic pressure exerted by the blood. The blood was allowed to flow directly from the blood vessel used into the flask, which was then swung in a vessel containing two to four litres of distilled water, and the dialysis was continued for five to twenty-four hours. The dialysate was then concentrated by evaporation to 50 to 100 c.c., and tested directly with the ethereal solution of β naphthalinsulphochloride, made alkaline according to the usual procedure by the addition from time to time of appropriate amounts of a normal solution of potassium hydroxide. The mixture was kept in the shaking apparatus for four to twenty-four hours. A positive reaction for amido acids was obtained in all cases but one in blood taken from fourteen different animals. The experiments were all made upon dogs, and the blood was collected either from the portal vein or from the carotid artery or jugular vein in animals that had been well fed, five to six hours after the meal, or in animals in a fasting condition, twenty-four to fifty hours after a meal.

Examination of the dialysate. — After a dialysis of five to six hours the dialysate was usually entirely clear; after a longer dialysis, how-

ever, it might become slightly milky, owing possibly to the diffusion and precipitation of some of the globulin.[1] On concentrating the dialysate on a sand bath a slight precipitate might coagulate out, especially if the dialysis had been continued for a long time. The filtrate from this precipitate was somewhat colored. An effort to clarify the solution by treatment with animal charcoal was unsuccessful, since this substance holds back the amido acids. With regard to the amido bodies in the blood it was found that they are removed practically completely by a dialysis of five to six hours. If after this time the flask is dialyzed against a new bulk of distilled water, the dialysate on concentration and examination in the usual way gives little or no indication of the presence of amido acids, while the first dialysate gives a distinct reaction. The condensed and filtered dialysate contains some sugar, but gives no indication of proteids when tested by the biuret or millon reagent. Inasmuch as preliminary tests with solutions of Witte's peptone showed that the collodium membrane is readily permeable to solutions of peptones and proteoses, the entire absence of a biuret reaction in the dialysate is a strong indication that no proteoses or peptones exist in the blood, even in the portal blood collected during the period of maximal absorption from the intestines. The question of the normal existence of peptones or proteoses in the blood, especially after a proteid meal, has given rise to much discussion (see accompanying paper on the proteids of the blood). The fact that a large proportion of the blood collected from the portal vein during full digestion and dialyzed against a membrane readily permeable to these substances, gives an entirely negative reaction to the biuret test, must be accepted as strong proof that the peptones and proteoses do not exist normally in the blood. When the concentrated dialysate was saturated with zinc sulphate, a small flocculent precipitate was obtained, but that this precipitate did not consist of peptones or proteoses or indeed of proteid of any character was shown by the fact that when filtered off and dissolved the solution failed to give a perceptible reaction with the biuret test.

The β naphthalinsulphochloride reaction. — As stated above, this reaction was positive in all cases except one in specimens of blood taken from fourteen different animals. The exceptional case was that of a dog in fasting condition, but it should be stated that in this instance the dialysate was shaken with the reagent for only two

[1] On two occasions hæmoglobin passed into the dialysate, owing to some imperfection in the membrane.

hours. The procedure used in testing was as follows. The concentrated dialysate was shaken with the ethereal solution of the reagent in distinct alkaline reaction for usually eight to nine hours. The ethereal solution was then removed by means of a separating funnel, and the aqueous solution after filtration was acidified with hydrochloric acid. A cloudy white precipitate was obtained, which if shaken out with ether seemed to dissolve completely. The ethereal solution thus obtained was separated and evaporated to dryness, and the residue was dissolved in water to which a trace of ammonia had been added; the excess of ammonia was removed by heating and the solution was filtered and precipitated by acid. The precipitate thus obtained settled to the bottom as a slightly colored layer which on examination under the microscope presented the appearance of minute droplets of an oily character. The precipitate could not be made to crystallize. Repeated solutions in alkaline liquids with precipitation by acid, and long standing at low temperature failed to cause the formation of crystals. Repeated precipitations from hot water or dilute alcohol failed to give a better result. In this respect the naphthalinsulphoamido compound differed from that obtained by other observers from the urine, and it would appear therefore that the amido acid indicated is not glycocoll. The compound obtained by von Bergmann from the blood behaved in a similar way as regards crystallization. If the first precipitate of the naphthalinsulphoamido compound obtained on acidifying the shaken mixture was allowed to stand instead of being shaken out with ether, it settled to the bottom of the beaker after some hours as a yellowish layer which adhered tightly to the bottom in the form of a smear. When the supernatant liquid was poured off and the smear was treated with ether, a portion dissolved. The portion soluble in ether, treated as described above, gave a pure white precipitate on acidifying which, however, could not be obtained in crystalline form. The portion not soluble in ether balled together as a gummy mass when rubbed with a rod and was deeply colored. This portion dissolved readily in dilute solutions of alkali, and on acidifying precipitated in the form of yellowish flocks which deposited finally as clumps of yellowish granules. This portion seemed to contain all of the pigment present in the original precipitate. The failure to obtain the naphthalinsulphoamido compound in crystalline form suggested the possibility that the reaction might be due to one of the more complex polypeptids. An effort was made therefore to hydrolyze this body. The concentrated dialysate was evaporated to

dryness on the water bath, and the residue was boiled with strong hydrochloric acid for eight hours with a reflux condenser. The solution was then evaporated to complete dryness on the water bath, taken up with water and treated with the β naphthalinsulphochloride. The precipitate obtained still deposited in oily drops, which could not be made to crystallize after repeated precipitations and exposure to low temperatures.

Comparison of portal and jugular blood. — The blood was examined from dogs in full digestion, five to six hours after a meal, and from others in a fasting condition, twenty-four to fifty hours after a meal, and in some cases the blood was withdrawn from the portal vein directly, in others from the jugular vein or carotid artery. The amount of material obtained as a naphthalinsulphoamido compound was small in all cases, and no weighings have been made as yet, but judged by the appearance of the precipitate, a larger yield was obtained from the portal blood taken during digestion than from the jugular blood, and a larger reaction from the blood of the fed animal than from that of the starved animal. In three cases out of four examined, however, a positive reaction was obtained from both the portal and the jugular blood of animals starved for fifty hours, and this fact would indicate that amido acids are constantly present in the blood. It is hoped later to obtain quantitative measurements. The fact that the amido acids seem to be increased after digestion, while no trace of peptones or proteoses can be dialyzed from the blood, lends support to the modern view that the proteids during digestion and absorption are completely hydrolyzed into their constituent amido acids, and that if any synthesis occurs, the products formed are either not present in the blood or are in a form which does not dialyze. It should be added that the concentrated dialysate from the blood gave always a precipitate with phosphotungstic acid in acid solution. Inasmuch as this reagent yields a precipitate so readily with dilute solutions of potassium or ammonium salts, a reaction of this kind is without significance in regard to the products of digestion unless the presence of organic bases can be detected by other means. An effort to obtain from this precipitate definite reactions for arginin gave negative results.

Amido acids in the lymph. — In one animal that had been well fed lymph was collected from the thoracic duct. Fifty cubic centimetres of this lymph were dialyzed against a litre of water for twenty-four hours. The dialysate was concentrated in the usual way, and, as in

the case of the blood, it contained sugar but gave no reaction with the millon or biuret test. Treated with the β naphthalinsulphochloride, it yielded a slight turbidity which settled to the bottom of the beaker as oily droplets which formed an adherent precipitate similar to that obtained from blood. The precipitate in this case was dissolved in barium hydrate, the excess of barium was removed by carbon dioxide; the solution was filtered, boiled, and again filtered. On acidifying a milky precipitate was obtained which on settling showed some tendency to crystallize in needles.

The osmotic pressure of the portal and the arterial blood.— In the dialysis as carried out in these experiments the blood showed considerable permanent osmotic pressure. If the blood was first heated to the point of coagulation, however, and then dialyzed, it showed little or no osmotic pressure, the membrane being so permeable to the salts and other readily diffusible constituents of the blood that the difference in concentration due to these substances was very quickly equalized by diffusion. It is evident, therefore, that the permanent osmotic pressure exerted by the blood is due to the contained proteids, or, as Reid supposes, to certain unknown substances, combined or mixed with the proteids, which are altered or precipitated when the proteids are coagulated. In these experiments the osmotic pressure was measured simply by the height to which the liquid rose in the tube attached to the dialyzing flask. It was observed that in the specimens of portal blood the liquid rose more promptly and to a greater height than in the case of the carotid blood. With the portal blood the height of the column of liquid was equal to 270 to 300 mm., while in the carotid blood it was from 200 to 220 mm. Inasmuch as the percentage of proteids in the portal blood is greater than in the carotid blood, this fact alone may explain the difference in permanent osmotic pressure. As measured by the lowering of the freezing-point, the concentration of the portal blood is considerably above that of the carotid blood, and the difference is distinctly increased during the period of digestion, as is shown by the following experiment.

Dog A. — Starved for twenty-four hours. Serum obtained from clots of blood, taken from the portal vein and the carotid artery. Serum entirely clear and slightly concentrated from standing.

Zero point for distilled water . . . . . = 2.989

Freezing-point of carotid serum (3 determinations) 2.370
2.369
2.369

2.369

Freezing-point of portal serum (3 determinations) 2.349
2.350
2.352
2.350

Δ of serum of carotid blood = 0.620°; of portal blood = 0.639°; difference = 0.019°.

Dog B. — Well fed; bled five hours after meal. Serum collected from clots was very milky — was centrifugalized.

Freezing-point of carotid serum (2 determinations) 2.360
2.355
2.357

Freezing-point of portal serum (2 determinations) 2.324
2.320
2.322

Δ of serum of arterial blood = 0.632°; of portal blood = 0.667°; difference = 0.035°.

Summary.

1. By means of dialysis through collodium membranes a substance or substances may be separated from the blood which give the reaction of amido acids with β naphthalinsulphochloride.

2. In the well-fed animal the amido acids are present in the blood of the portal vein in larger amounts than in the general systemic blood. Even after a starvation of fifty hours, however, a positive reaction may be obtained from the blood.

3. Lymph collected after a meal and treated in the same way gives a positive reaction.

4. Although the collodium membrane is readily permeable to peptones and proteoses, no indication of the existence of these substances can be obtained from dialyzing portal blood collected during the period of maximum absorption.

5. Owing probably to its larger percentage in proteids, the portal blood exhibits a permanent osmotic pressure larger than that shown by blood collected from the general circulation.

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